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AN 2002008965 MEDLINE
DN 21237598 PubMed ID: 11338924
TI Satellite DNA-based artificial chromosomes for use in gene therapy.
AU Hadlaczky G
CS Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, PO Box 521, Hungary.. hgy@nucleus.szbk.u-szeged.hu
SO Curr Opin Mol Ther, (2001 Apr) 3 (2) 125-32. Ref: 33
Journal code: 100891485. ISSN: 1464-8431.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
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LA English
FS Priority Journals
EM 200112
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L7 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2002 ACS
D
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AN 2000:716318 CAPLUS
DN 134:232422
TI Satellite DNA-based artificial chromosomes-chromosomal vectors. Reply to Comments
AU Brown, William R. A.
CS Institute of Genetics, University of Nottingham, Nottingham, UK
SO Tr nds in Biotechnology (2000), 18(10), 403
CODEN: TRBIDM; ISSN: 0167-7799
PB Elsevier Science Ltd.
DT Journal
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L7 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2002 ACS

AN 2000:716317 CAPLUS
DN 134:232421
TI Satellite DNA-based artificial chromosomes-chromosomal vectors. Comments
AU Perez, Carl; de Jong, Gary; Drayer, Jan
CS Chromos Molecular Systems Inc., Burnaby, BC, Can.
SO Trends in Biotechnology (2000), 18(10), 402-403
CODEN: TRBIDM; ISSN: 0167-7799
PB Elsevier Science Ltd.
DT Journal
LA English
L7 ANSWER 6 OF 9 MEDLINE DUPLICATE 2

AN 2000493348 MEDLINE
DN 20297740 PubMed ID: 10841045
TI Generation of transgenic mice and germline transmission of a mammalian

Enzymes show a delicate and complicated balance of all the factors that help determine the characteristics of the biocatalysts, and molecular modeling techniques can be extremely helpful in elucidating these interconnected factors. In the previously cited case of the thermostable mutant of subtilisin E, for example, the use of an all atom representation of the protein, its mutant and the solvent, together with the application of molecular dynamics to study the time evolution of the system, have allowed us to obtain a clear picture of enthalpic and enthropic factors that determine enzyme stability and activity[11]. It was revealed that the penetration of solvent into the hydrophobic core of the native protein, which leads to unfolding, involves one region of the protein in particular. A semiquantitative definition of flexibility is also given, helping to obtain a clear picture of the factors that determine the behaviour of subtilisin E and of its thermophilic homologue[11].

Thus, the use of directed evolution (coupled with suitable screening methods) and molecular modeling techniques can become an extremely important factor in the elucidation of the relationship between activity and stability in enzymes. The experimental results could be used by the modelers to increase their knowledge about the reasons of protein stability and to design better catalysts – enzymes that couple high activity and high stability – which would be a major achievement in the area of applied biocatalysis.

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Satellite DNA-based artificial chromosomes – chromosomal vectors

Letter

We were intrigued by a recent review[1] in which Brown *et al.* described two approaches to developing vectors that have the properties of chromosomes. We would like to bring attention to the existence of a third chromosome-based vector technology – satellite-DNA-based artificial chromosomes (SATACs; Refs 2,3) – which has been extensively reviewed[4,5].

In the first protocol that Brown described – cellular-mediated chromosome assembly – chromosomes were assembled from transfected alphoid, telomeric and marker DNAs (Refs 6,7). The limitations of this approach are that: (1) there is no predictable relationship between input DNA and assembled chromosomes; (2) rearrangements frequently occur when the transfected DNA fails to form neochromosomes; (3) the neochromosomes form at frequencies of $\sim 5 \times 10^{-5}$; and (4) neochromosomes have been shown to form only in HT1080 human fibrosarcoma cells.

The second protocol involves fragmenting natural human chromosomes using telomere-directed breakage to generate minichromosomes[8,9]. Transgenic animals have been generated with human–murine minichromosome chimeras[9], with fragmented human chromosomes[10–12], and recently with human small accessory chromosomes (SACs) (P. Marynen *et al.*, unpublished data). The primary limitation of this protocol is the inefficient and tedious transfer of these minichromosomes from one cell type to another by microcell fusion.

A third approach was omitted from the review by Brown *et al.* This approach uses neochromosomes formed by the *de novo* amplification of pericentric heterochromatin, SATACs (Refs 2–4). Exogenous plasmid DNA is introduced into the vicinity of mouse[2,3] and human[13] pericentric heterochromatin, thus forming *de novo* dicentric chromosomes as the result of the amplification of endogenous pericentric heterochromatin and marker sequences. Ensuing breakage generates chromosomes that range from 10 to 360 megabases. The murine chromosomal vector has been transferred to hamster, human and bovine cells, and is transmitted as a discrete chromosome with segregation efficiencies per generation between 99.0% and 99.8% (Ref. 14). These chromosomes are largely composed of repetitive sequences, thus enabling high-throughput isolation using flow cytometry[15]. Transgenic mice have been generated using the unique process of microinjecting isolated SATACs into the pronuclei of fertilized murine oocytes[16]. This extra

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chromosome has been transmitted through the germline over three generations and has been maintained as a discrete chromosome for over 14 months in lymphocytes (D.O. Co *et al.* unpublished data). Recently, transgenic founder mice and their progeny have been generated that carry a SATAC with a 1.0-megabase payload.

We have extended our studies on chromosome delivery beyond microcell fusion. SATACs isolated using flow cytometry have been transferred intact to other cell types by electroporation, microinjection and lipid-mediated techniques – generating stable transformants at efficiencies exceeding 10^{-2} (de Jong *et al.*, unpublished data).

We believe that by using SATACs to deliver large DNA payloads in an efficient, safe and stable manner, we have taken the first steps in developing an artificial chromosome as an 'ideal vector'.

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Response from Brown

Letter

We thank Perez *et al.* for their interest in our recent review and for providing the opportunity to discuss artificial chromosomes in more detail. In our review, we argued that artificial chromosomes are potentially interesting because they should enable some of the *cis*-acting sequences that are necessary for chromosome function to be identified and because they might enable long tracts of genomic DNA to be introduced into the genomes of livestock or crop plants. We argued that both of these uses need artificial chromosome vectors of defined structures and a technology that allows precise modification of the artificial chromosome molecules. Satellite-DNA-based artificial chromosomes (SATACs) do not have molecularly defined structures; we did not discuss SATACs in this review because we doubt that they will ever be useful either for addressing questions of basic biological interest or for biotechnological purposes. Perez *et al.* have emphasized the potential value of their SATACs as vectors. However, the genes present on SATACs have not, to our knowledge, been identified. Thus, given the current difficulties in Europe and the US involving crop plants modified with a single gene, we find it hard to see how regulators will accept animals carrying a chromosome that might bear many undefined genes. Perez *et al.* report unpublished data that describes efficient transfer of SATACs between cells. This point is potentially interesting because it bears upon a technical problem that is common to all the approaches towards artificial chromosome construction and we look forward to seeing the data in published form.

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Letters to the Editor

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